Biocriteria Implementation Procedures

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Executive Summary

This document sets forth implementation procedures for the proposed narrative biocriteria water quality standard. The document provides a narrative biocriteria statement and explains ADEQ's objective basis for determining compliance with this new standard.

The proposed biocriterion for wadeable, perennial streams of Arizona consists of a narrative statement, associated Index of Biological Integrity scores for cold and warm water streams, and a statement of applicability to various water body types. The following narrative statement is the proposed language for the narrative biocriterion.

"*A wadeable perennial stream shall support and maintain as community of organisms having a taxa richness, species composition, tolerance, and functional organization comparable to that of a reference stream in Arizona.*"

ADEQ will determine compliance with the narrative biocriterion through bioassessment and comparison of the bioassessment results to either a cold water or warm water Arizona Index of Biological Integrity. The biological integrity of a wadeable, perennial stream, as determined by the applicable Arizona Index of Biological Integrity (IBI), is protected at or above the 25th percentile of reference condition. An IBI score that is at or above the 25th percentile of reference condition meets the biocriterion. An IBI score that falls below the 10th percentile of reference condition violates the biocriterion. An IBI score that falls between the 10th and 25th percentile of reference condition is determined to be inconclusive and a verification bioassesment is required to determine whether there is a violation. If the verification sample IBI score falls below the 25th percentile, the biocriterion is violated.

In effect, a violation of the biocriteria standard occurs two ways: 1) a violation occurs when an IBI score for a study site is less than the 10th percentile of reference threshold value, and 2) a violation occurs when an IBI score for a study site is between the 10th and 25th percentile of reference threshold values and a verification sample also falls below the 25th percentile of reference threshold value.

The narrative biocriterion applies only to perennial, wadeable stream segments with either a warm or cold water aquatic life designated use. ADEQ has not characterized reference conditions for other water body types and does not have IBIs for them. The narrative biocriterion does <u>not</u> apply to large rivers, effluent dependent waters, ephemeral waters, intermittent waters, lakes, or wetlands.

ADEQ will determine compliance with the narrative biocriterion based on a macroinvertebrate sample collected from a wadeable, perennial stream with fast-flowing riffle or run habitat with heterogeneous substrate that is collected during the appropriate spring index period. The warm water IBI will apply to perennial, wadeable streams found at <5000' elevation and the cold water IBI will apply to perennial, wadeable streams found at >5000' elevation. ADEQ methods for biological sample collection and data analysis must be followed to compare bioassessment results to these macroinvertebrate-based IBIs.

Acknowledgments

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Introduction

The Environmental Protection Agency (USEPA) has been urging states to develop bioassessment methods and incorporate biocriteria into surface water quality standards since the *Rapid Bioassessment Protocols for Use in Streams and Rivers* were published (Plafkin et al, 1989). Biocriteria provide a direct measurement of biological integrity, one of the three objectives identified in the Clean Water Act. Bioassessment data are important for measuring the attainment of water quality standards for the protection of aquatic life because they utilize surveys of resident living organisms. Bioassessment data provide a clear picture of whether a water body is meeting its designated aquatic life use and can validate whether existing water quality criteria for toxic chemicals and physical parameters are adequately protecting that use (USEPA, 2002a).

Biological indicators such as macroinvertebrates, algae and fish integrate the cumulative effects of different stressors such as excess nutrients, toxic chemicals and excessive sediment over time. The biology provides a more reliable assessment of long-term ecological changes in the condition of a water body than do rapidly changing water chemistry measurements or laboratory toxicity tests. As such, the biota provides a unique indicator of ecological health, unlike any other measurement. The USEPA recommends that states use biological assessment data as a core indicator for making aquatic life use determinations.

Background

The concept of biological integrity is embedded in §101(a) of the Clean Water Act. The primary objective of the Clean Water Act is "to restore and maintain the chemical, physical, and biological integrity of the nation's waters." Section 101(a)(2) of the Act sets forth the national goal that "…wherever attainable, an interim goal of water quality which provides for protection and propagation of fish, shellfish, and wildlife and provides for recreation in and on the water be achieved." The intent of this statement is to protect not only what is currently living in our waters but also to provide for maintenance of viable, reproducing populations.

Biological integrity is commonly defined as "the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity and functional organization comparable to that of the natural habitat of the region" (Karr and Dudley, 1981). This concept refers to the natural assemblage of indigenous organisms that would inhabit a particular area if it had not been affected by human activities. The naturally occurring biological diversity becomes the primary reference condition used to measure and assess the attainment of aquatic life goals. Arizona's narrative biocriterion at A.A.C. R18-11-108(E) is a restatement of Karr's definition of biological integrity. ADEQ defines "reference condition" in the surface water quality rules at A.A.C. R18-11-101(33) as "a set of ecological measurements from a population of relatively undisturbed water bodies within a region that establish a basis for making comparisons of biological condition among samples."

ADEQ began a bioassessment program in 1992 following the USEPA *Rapid Bioassessment Protocols for Use in Streams and Rivers* (Plafkin et al, 1989; Barbour et al., 1999), which provided guidance for development of biological monitoring and assessment procedures. Standard operating procedures for macroinvertebrate monitoring in perennial, wadeable streams of Arizona and for laboratory processing and taxonomic identification were established and recently updated in the *Biocriteria Program Quality Assurance Program Plan* (ADEQ, 2005). A statewide reference site monitoring network was established to develop indexes of biological integrity as the macroinvertebrate bioassessment tool. Reference site monitoring continues in two watersheds each year.

A classification analysis was performed on the statewide macroinvertebrate dataset to identify regions of statistically different macroinvertebrate communities across the state (Spindler, 2001). Elevation-based regions were the result of the classification analysis, consisting of two broad macroinvertebrate regions and community types:

- A warm water community located below 5000 feet of elevation
- A cold water community located above 5000 feet of elevation

All wadeable, non-effluent dependent, perennial streams located in these regions, with some exceptions (see section on applicability) are predicted to have the same general macroinvertebrate community type. Indexes of Biological Integrity (IBI) were developed for both a warm water community and a cold water community type, using Arizona's statewide network of reference site data (Gerritsen and Leppo, 1998; Leppo and Gerritsen, 2000). Background information about reference conditions and the development of the IBIs is presented in the Biocriteria Technical Support document (ADEQ, 2006).

Narrative Biocriteria Standard

The proposed biocriteria standard for wadeable, perennial streams of Arizona at R18-11-108(E) and the biocriteria implementation procedures at R18-11-108.01 consists of: a) the narrative biocriterion, b) a statement of applicability, c) rules explaining how the biocriterion is met, and d) associated IBI scores for cold and warm water streams. ADEQ proposes to add R18-11-108.01 to the surface water quality standards rules in the 2008 triennial review. The following narrative statement is the proposed language of the narrative biocriterion:

> "*A wadeable perennial stream shall support and maintain a community of organisms having a taxa richness, species composition, tolerance and functional organization comparable to that of a reference stream in Arizona.*"

Applicability

The narrative biocriterion applies only to wadeable perennial streams with either a cold or warm water aquatic life designated use. The Arizona Water Quality Standards (A.A.C. Title 18. Chapter 11) currently list four aquatic life uses: aquatic life (cold water), aquatic life (warm water), aquatic life (ephemeral), and aquatic life (effluent-dependent). Developing biocriteria for cold and warm water perennial streams has been the priority because of the diverse aquatic and terrestrial life they support and because aquatic communities of perennial streams have predictable community structure and function. ADEQ may revise the biocriteria standard and develop implementation procedures for other water body types as new scientific information becomes available.

Several stream conditions must be met in order to apply the Arizona Indexes of Biological Integrity (IBI). The sampling site conditions and collection time frame must meet the following conditions:

- **Wadeable,**
- **Perennial,**
- **Contain fast-flowing riffle or run habitat,**
- **Are not dominated by bedrock or travertine,**
- **Sampled during the spring index period** (April-May for warm water streams and May-June for cold water streams).

These conditions are important for determining that the study samples are collected from streams that are similar to the reference stream sites, and to prevent sample collections from habitats or during time periods when conditions would lead to a false finding of impairment. **Wadeable** means no deeper than can be safely waded across when collecting samples. **Perennial** refers to stream segments which flow continuously throughout the year (excluding effluent dependent waters). **Riffle habitat** refers to the portions of streams where moderate velocities and substrate roughness produce moderately turbulent conditions which break the surface tension of the water and may produce white water (Bain and Stevenson, eds. 1999). **Run habitat** refers to segments of streams where there is moderate velocity water, but non-turbulent conditions which do not break the surface tension of the water and do not produce white water (Bain and Stevenson, eds.1999).

"Not dominated by bedrock or travertine"means that the stream bottom material consists of less than 50% bedrock or travertine within the reach. Streams that are bedrock- or travertine-dominated have aquatic communities which exhibit limited taxa richness and loss of structure and function when compared with reference conditions.

The **spring index period** is defined as a period of time following winter runoff in which baseflow conditions will be found in most streams. Baseflow conditions generally are achieved post-winter runoff in the desert streams in April-May and in mountain streams in May-June. A period of 4 weeks post-bankfull flood condition is generally required prior to macroinvertebrate sampling, even during the spring index sampling period. Hydrologic conditions are checked in the office prior to a site visit and field conditions are documented on the SEM Field Form for

Macroinvertebrate Sample Collection (Appendix A) in the field prior to sampling to confirm that sampling is occurring during the appropriate sample collection conditions.

The proposed narrative biocriterion will not apply to the following water body types until ADEQ adopts IBI scoring thresholds specific to these types of waters:

- **Effluent dependent waters**
- **Intermittent streams**
- **Ephemeral streams**
- **Large rivers**
- **Lakes**
- **Wetlands**

Determining a Biocriteria Standard Violation

ADEQ will determine compliance with the narrative biocriterion from bioassessment results and comparison to the applicable Arizona Index of Biological Integrity (IBI) for warm and cold water streams. ADEQ will consider the biological integrity of a wadeable, perennial stream to be adequately maintained and supported when a bioassessment result, as measured by an IBI score, is at or above the 25th percentile of reference condition. An IBI score that is at or above the 25th percentile of reference condition meets the biocriterion. An IBI score that falls below the 10th percentile of reference condition violates the biocriterion. An IBI score that falls between the 10th and 25th percentile of reference condition is inconclusive and a verification sample is required to determine whether there is a violation. If the verification IBI score is at or above the 25th percentile, the biocriterion is met. If the verification IBI score is less than the 25th percentile, the biocriterion is violated. The IBI scores in Table 1 comprise the thresholds ADEQ proposes to use to implement the narrative biocriterion:

Table 1. **Macroinvertebrate Index of Biological Integrity thresholds for wadeable, perennial streams of Arizona**

Macroinvertebrate bioassessment result	**Index of Biological Integrity Score**	
	Cold water	**Warm water**
Greater than the 25th percentile of reference condition	≥ 52	≥ 50
Greater than the 10th and less than the 25th percentile of reference condition	46 – 51	40 – 49
Less than the 10th percentile of reference condition	≤ 45	≤ 39

ADEQ's biological data collection, taxonomy and analysis procedures must be followed in order to apply the Arizona Index of Biological Integrity. ADEQ biological field sampling, laboratory methods and analysis procedures are provided in Appendix A and B. These procedures are published in the *Manual of Procedures for the Sampling of Surface Waters* (Lawson, 2005).

Implementation of Biocriteria and Associated Implementation Procedures in AZPDES Permits

The narrative biocriterion at A.A.C. R18-11-108(E) and the numeric IBI thresholds for wadeable, perennial streams in A.A.C. R18-11-108.01(C) are not intended to be used as a basis for calculating numeric water quality based effluent limits (WQBELs) in AZPDES permits. ADEQ may develop AZPDES permit conditions which require ambient water quality monitoring, including bioassessment, to assess ambient stream conditions and to confirm the effectiveness of point source discharge controls or best management practices.

Use of Biocriteria for Water Quality Assessment and Identification of Impaired Waters Under §303(d) of the Clean Water Act.

CWA §305(b) water quality assessments and impaired waters identification under CWA §303(d) will be based on the narrative biocriterion at R18-11-108(E) and the biocriteria implementation procedures contained in R18-11-108.01. A single macroinvertebrate sample may be used to determine the attainment status of the A&Wc or A&Ww designated uses. There are several reasons why a single sample is appropriate for bioassessment.

- A single macroinvertebrate sample represents long-term conditions because macro-invertebrates reside in the stream year-round.

- The sampling method is sufficiently rigorous that it limits the amount of variability in sample collection. Precision of the indexes is high, with a standard deviation of only 7 points on a 100 point scale, for repeat visits across years in the warm water dataset (Gerritsen and Leppo, 1998).

- The Indexes of Biological Integrity are robust measures of the structure and function of the macroinvertebrate community and limit the variability associated with a single metric.

- The reference condition, upon which the indexes are constructed, consists of replicate samples over a 5-year period and across regions of the state. Reference conditions are a composite by which to compare study reaches, thereby limiting the variability associated with individual reference sites.

There is a high level of certainty about making an assessment of attainment of the aquatic life use at the 25th percentile of reference condition. Similarly, there is a high level of certainty that wadeable, perennial stream is biologically impaired when the IBI score is less than the 10th percentile of reference condition. However, there is less certainty about making a determination of "impaired" when IBI scores fall just below the 25th percentile. To avoid having false positive assessments of perennial streams that are biologically impaired, a verification sample will be

required when the IBI score falls within the zone of uncertainty between the 10th and 25th percentiles of reference condition (Table 2, Figure 1, Figure 2).

Table 2. Assessments based on ADEQ macroinvertebrate IBI scores

Macroinvertebrate bioassessment result	Index of Biological Integrity Score		Assessment
	Cold water	Warm water	
Greater than the 25th percentile of reference condition	≥ 52	≥ 50	Attaining
Between the 10th and 25th percentile of reference condition	46 – 51	40 – 49	Inconclusive
Less than the 10th percentile of reference condition	≤ 45	≤ 39	Impaired

When the IBI score for the verification sample falls below the 25th percentiles, we have sufficient evidence that the biological community is degraded and the study reach will be assessed as impaired for the aquatic life use. When the IBI score for the verification sample is greater than the 25th percentile confidence interval, there is sufficient evidence that the community is improving, therefore the study reach will be assessed as attaining the aquatic life use.

Identification of Impaired Waters

The same criteria that are used for determining a violation of the narrative biocriterion will be applied to identify biologically impaired waters for purposes of CWA §303(d). A water body will be placed on the §303(d) list of impaired waters when the IBI score is less than the 10th percentile for a single sample. A water body will be placed on the §303(d) list when an initial IBI score is found to be inconclusive (between the 10th – 25th percentiles) and a verification sample is found to be less than the 25th percentile. The placement of a water body on the §303(d) list and delisting will be based on the most recent bioassessment. After a water body is assessed as biologically impaired, the water body can be de-listed when the results of a subsequent bioassessment indicates that the IBI score is greater than the 25th percentile of reference condition.

Identification of the stressor causing impairment of the biological community will be conducted as part of a TMDL investigation. Detailed methods for conducting the stressor identification process are described in the USEPA's *Stressor Identification Guidance Document* (USEPA, 2000).

Definitions:

Bankfull elevation: The channel elevation at which an annual high flow event with a 1-2 year return interval occurs. Bankfull elevation is estimated using regional curves and field measurements and several field indicators are identified to determine if recent high flows have occurred. These indicators are listed in the ADEQ Stream Ecosystem Monitoring field forms.

Bankfull flow means the discharge level in cubic feet per second, which corresponds to the annual high flow event having a return interval of 1-2 years. The "high flow checklist" in the SEM field forms is used to determine in the field whether a high flow has recently occurred.

Biological assessment (Bioassessment): An evaluation of the biological condition of a surface water using biological surveys of the resident living organisms.

Biological criteria (Biocriteria): Narrative expressions or numeric values that describe the reference biological integrity of aquatic communities inhabiting waters of a given designated aquatic life use.

Biological integrity (Biointegrity): The capacity of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of the natural habitat of the region.

Effluent-dependent water: A surface water that consists of discharges of treated wastewater that is classified as an effluent-dependent water by the Director under R18-11-113. An effluent-dependent water is a surface water that, without the discharge of treated wastewater, would be an ephemeral water.

Ephemeral water means a surface water that has a channel that is at all times above the water table, and that flows only in direct response to precipitation.

Index of biological integrity means a multimetric tool used for assessing the condition of a biological community.

Intermittent surface water means a stream or reach of stream that flows continuously only at certain times of the year, as when it receives water from a spring or from another surface source, such as melting snow.

Macroinvertebrates are invertebrate animals that are large enough to be seen with the naked eye and have no backbone or spinal column; such as insects, snails and worms.

Metric means a characteristic of the biota which changes in some predictable way with increased human disturbance.

Perennial surface water means a surface water that flows continuously throughout the year.

Regional reference condition means a set of ecological measurements from a population of relatively undisturbed water bodies within a region that establish a basis for making comparisons of biological condition among samples.

Riffle habitat refers to the portions of streams where moderate velocities and substrate roughness produce moderately turbulent conditions which break the surface tension of the water and may produce whitewater.

Run habitat refers to segments of streams where there is moderate velocity water, but non-turbulent conditions which do not break the surface tension of the water and do not produce whitewater.

Spring index period means the time period following winter runoff and snowmelt, when baseflow conditions generally occur in Arizona streams. For macroinvertebrate sampling purposes, spring index period is defined as April 1-May 31st for warm water streams and May 1 – June 30th for cold water streams.

Study reach: A macroinvertebrate sample is collected over a stream segment that is 2 meander lengths long or a minimum of 100 meters long in larger streams. It represents biological integrity of the assessment unit within which it is collected.

Wadeable means no deeper than can be safely waded across when collecting samples. ADEQ recommends sampling in streams that are flowing at velocities and depths whose quotient is less than 9 (eg. Velocity <4.5ft/s x 2 ft deep).

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Appendix A: SEM Field Form, Macroinvertebrate Sample Collection

Decision Criteria for Sampling Macroinvertebrates

Site Location:
Date/Time:
Field Crew:

The target stream habitat for collecting macroinvertebrates must be wadeable, perennial, contain riffle or run habitat, must contain heterogeneous substrates, and must be sampled during the spring index period. Spring index period is April – May for warm water streams and May – June for cold water streams. Use the following specific decision criteria to determine whether to collect a macroinvertebrate sample. Circle the action taken regarding whether a sample was collected. Where you have found the stream conditions to be inappropriate for macroinvertebrate sampling, record a comment indicating the rationale for not collecting.

Parameter	Condition	Action to Take
Hydrologic conditions	Baseflow conditions are occurring and it is approximately 4 or more weeks after a bankfull flow event. *	Collect macroinvertebrates
	A bankfull or greater magnitude flow event has occurred within 4 weeks of site visit. Or extreme high flow events have occurred resulting in deep scouring of the streambed and benthic community such that the macroinvertebrate community will not recover within the spring index period.	Do not collect macroinvertebrates
	Extended drought conditions have reduced flow from previously perennial condition to pools only or stagnant wetland habitat.	Do not collect macroinvertebrates
Substrate Type	A substrate consisting of a mixture of some of the following particle sizes is the target condition: boulder, cobble, gravel, sand, clay, silt, bedrock.	Collect macroinvertebrates
	Streams which have substrates dominated (consisting of >50% of that substrate type) by bedrock or travertine are considered non-target conditions.	Do not collect
Waterbody Type	The target waterbody type is a flowing stream with riffle or run (erosional) habitats present.	Collect macroinvertebrates
	We do not have methods developed for the following waterbody types and are not sampling them at this time: Effluent dependent streams, wetlands, ephemeral streams, lakes, seasonally intermittent streams.	Do not collect

Comments: (indicate rationale for not collecting macroinvertebrate sample, if different from the above descriptions)

* Identification of bankfull and high flow elevation in the field: Using known watershed area, use appropriate Regional Curve and field bankfull indicators to estimate bankfull elevation. Look for debris lines and other high flow markers as an indicator of the most recent high flow stage. This procedure is explained in more detail and a copy of the regional curves is provided in the ADEQ Habitat Assessment Procedures (2005).

Appendix B: Procedures for Biological Sampling of Streams and Rivers

Lawson, L.L., ed. 2005. Macroinvertebrate Sampling and Analysis Procedures, Section 3, Part A, *in A Manual of Procedures for the Sampling of Surface Waters*. Arizona Department of Environmental Quality, TM05-01. Phoenix, AZ

3.15 Biological Sampling of Streams and Rivers

3.15.1 Macroinvertebrates

Macroinvertebrate sampling is conducted to assess the biological integrity of perennial, wadeable streams. ADEQ has developed bioassessment tools in the form of Indexes of Biological Integrity (IBI) along with habitat evaluations for this purpose. There are two IBIs, the Warm water IBI and Cold water IBI. The procedure for calculating the IBIs is found in Section 3.15.2 and in the Biocriteria Program QAPP (ADEQ, 2001). Guidance for performing habitat evaluations, needed to determine causes and sources of impacts, is presented in Section 3.17. This procedure presents the field methods used for collecting macroinvertebrates, the initial step in conducting a bioassessment.

Equipment required: D-frame net fixed with a 500 micron mesh net (Figure 3.15.1a), forceps, a large bucket, a metal sieve having a 500 Φm mesh, a white dissecting tray, 37% formaldehyde, and 99% isopropyl alcohol.

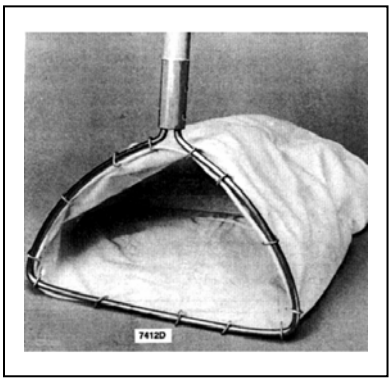

Figure 3.15.1a D-frame net with 500 micron mesh net.

3.15.1.1 Site selection

The study reach length should be one of the following: 1) 25 times the bankfull width of the stream in wadeable streams, or a minimum of 100 meters in larger streams, or 2) long enough to encompass 2 meander lengths of the stream and multiple riffles in which to produce a composite sample, but not less than 100 feet in length. For a definition of bankfull, see the Rosgen stream type identification section of the habitat assessment protocol, Section 3.17.3. The study reach should be selected to represent typical habitat conditions found in the larger stream segment. The study reach length should begin at the top of a riffle or run and end at the bottom of a riffle or run.

If the reach is to be used as a reference or background reach, the following general criteria must be met:

- The site must be accessible within a 2-hour walk or 3-4 miles from the nearest four wheel drive road,
- No known discharges upstream,

- ❖ No major impoundments upstream.
- ❖ No human caused channel alterations at the site; e.g. diversions, dredge and fill projects,
- ❖ At least 0.5 miles downstream of road crossings,
- ❖ The site should be perennial. The indicators for perennial condition are likely to be the presence of fish, univoltine insects, and healthy unstressed riparian plants.
- ❖ The site should be free of local land use impacts,
- ❖ There should be no recorded violations of pH or dissolved oxygen water quality standards, and
- ❖ The Habitat Assessment Index score should be greater than 14 (see Section 3.17.2.6).

3.15.1.2 Sample Collection Information

Biocriteria research efforts have focused on perennial, wadeable streams to date. Sample collection methods have been developed and refined only for this waterbody type at this point. The following sampling conditions and time frames must be met in order to collect macroinvertebrates for ADEQ bioassessment purposes. A stream reach must be:

- Wadeable
- Perennial
- Contain fast-flowing riffle or run habitat
- Not bedrock or travertine dominated
- Sampled during the spring index period (April-May for warm water streams and May-June for cold water streams).

Wadeable means no deeper than can be safely waded across when collecting samples. **Perennial** refers to stream segments which flow continuously throughout the year (excluding effluent dependent waterbodies). **Riffle habitat** refers to the portions of streams where moderate velocities and substrate roughness produce moderately turbulent conditions which break the surface tension of the water and may produce whitewater (Bain and Stevenson, eds. 1999). **Run habitat** refers to segments of streams where there is moderate velocity water, but non-turbulent conditions which do not break the surface tension of the water and do not produce whitewater (Bain and Stevenson, eds.1999).

Bedrock or travertine dominated means that 50% or more of the stream bottom material consists of bedrock or travertine. Streams that are bedrock or travertine dominated have aquatic communities which exhibit limited taxa richness and loss of structure and function when compared with reference conditions.

The **spring index period** is defined as a period of time following winter runoff in which baseflow conditions will be found in most streams. Baseflow conditions generally are achieved post winter runoff in the desert streams in April-May and in mountain streams in May-June. A period of 4 weeks post-bankfull flood condition is generally required prior to macroinvertebrate sampling, even during the spring index sampling period. Hydrologic conditions are checked in the office prior to a site visit and field conditions are documented on the SEM Field Form for Macroinvertebrate Sample Collection in the field prior to sampling to confirm that sampling is occurring during the correct sample collection conditions.

Macroinvertebrate samples are not collected when the following conditions occur:

- A bankfull or greater magnitude flow event has occurred within 4 weeks of site visit or when extreme high flow events have occurred, resulting in deep scouring of the streambed and benthic community such that the macroinvertebrate community will not recover within the spring index period.
- Extended drought conditions have reduced flow from previously perennial condition to pools only or stagnant wetland habitat.
- Stream substrates are dominated (consisting of >50% of that substrate type) by bedrock, or travertine are considered non-target conditions.

Macroinvertebrate samples should be collected before pebble counts and before any disturbance to the stream channel by investigators. The collection begins at the downstream end of the assessment reach and proceeds upstream.

A macroinvertebrate sample consists of a three-minute timed composite sample from kick samples collected from three riffle habitats within the study reach. The target sampling area is approximately one square meter per each one minute sample. Select 3 or more riffles which represent the variety of substrate sizes, velocities, depths, and habitats found within the reach. Collect one-minute timed samples from each of three habitats or divide the time as needed among the variety of habitats. If three good-sized riffles are not available to be sampled, spread the three minute sample time over whatever riffle/run areas are available.

3.15.1.3 Sample Collection

Once the sampling sites within the reach have been selected, the first sample should be at the lowermost riffle. The procedure is as follows:

1. Fill a bucket half full with stream water.

2. Place the D-frame net on the stream bed in the path of flowing water, and agitate a one square meter area of substrate vigorously for one minute by kicking or hand turning rocks to collect dislodged material. Sample as much variation of the flow and substrate as possible including large and small substrates.

3. Deposit the contents of the net into the bucket. At this point there is no need to pick the net clean.

4. Repeat the sampling procedure for the second and third riffles. After the last riffle, use forceps to remove organisms attached to the D-frame net. Before leaving the site, the D-frame dip net, bucket, and sieve should be rinsed and scrubbed with a brush to dislodge small invertebrates, egg masses, and organic material, so that it is not transferred to the next site.

5. Swirl the contents of the bucket and pour the non-sediment portion into a 500 μm mesh sieve.

6. Add water again to the bucket, swirl and pour the contents into the sieve. Repeat this procedure several times until all insects and organic debris are emptied and only sediment remains.

7. Dump the remaining sediment into a dissecting tray and search the sediment for any remaining organisms, especially cased Trichoptera, snails, and freshwater clams and then discard the remaining sediment.

8. Gently, squeeze the sample to remove excess water from algae laden samples. Using a plastic spoon or hands, gently dispense the sample from the sieve into a wide mouth, one-liter sample jar. Fill the jar half to three-quarters full. If additional sample remains in the sieve, use an extra jar to contain it. Rinse any leftover material in the sieve into a corner and spoon out as much as possible. Check the sieve for any remaining animals. If the entire sample does not fit into one jar, then add the remainder to a second jar. If the sample will not fit into two jars, then field split the sample.

9. To perform a one-half field split, evenly spread the entire sample in a white dissecting tray and divide the sample with your hands into two equal portions. Place one half of the sample into the two sample jars and discard the other half into the stream. Note on the field form that the sample was "field split ½". A quarter split can be performed if a half-split still provides too much sample material to fit in two jars.

10. Place label(s) (see Section 3.15.3.2) in the jar(s), add enough 99% isopropyl alcohol to cover the sample material by about 1 inch.

11. Seal the jar(s), and affix a second label to outside of the jar(s).

3.15.1.4 Sample Labeling

Each macroinvertebrate sample should have two identification labels penciled on "write-in-the-rain" paper: one placed inside the jar, visible from the outside, and one affixed to the outside of the jar, attached with clear plastic tape. If more than one jar is used for a sample, put jar numbers on all labels (e.g., 1 of 2, 2 of 2). Each tag should have the following information at a minimum:

Waterbody name
Site code number
Habitat sampled (riffle)
Date
ADEQ and collectors' initials

3.15.1.5 Preservation and Storage

After samples have been preserved with 99% isopropyl alcohol, samples should be placed in an ice chest with ice to cool the sample. This prevents overheating and degradation of the sample, and prevents fumes from developing inside truck camper shells.

Samples should be stored in a cool environment and within flammable storage areas in the laboratory prior to shipping to the laboratory.

3.15.1.6 Chain of Custody

To complete the Chain of Custody, samples shall be locked in field trucks when sampling personnel are away from the truck. Sample jars shall be placed in the large, locked flammable cabinet in the equipment storage area of the ADEQ laboratory for storage, prior to shipping. The use of tamper-evident tape on shipping boxes to prevent tampering with samples during shipping is required. A Chain of Custody form will accompany the samples during shipment.

3.15.1.7 Sample Preparation for Shipping to Taxonomy Laboratory

1. Drain sample over a 500 micron sieve and large funnel into a waste container (e.g. 5-gallon isopropanol carboys) under a fume hood and place the sample back into the sample jar along with the correct interior label. Insure that all specimens have been picked off the sieve and placed in sample container, then cap tightly. Rinse and scrub the sieve with a brush prior to draining the next sample.

2. Wrap jars in bubble wrap and place in a heavy duty garbage bag with an absorbent sheet, inside a shipping box. ADEQ uses ADOT approved boxes that are 14x10x10", single-wall construction, edge Crush Test of 32 lbs/inch, gross weight limit of 65 lbs. The bubble wrap prevents shock and deterioration of the sample specimens. Close and seal the garbage bag. Place the Laboratory address on a sheet of paper inside the box, along with an inventory of samples. Seal the box with tamper-proof tape to continue chain of custody while samples are in transit. Mark boxes with ▪this side up▪ arrows to further prevent leakage and protect samples.

3. Ship boxes via commercial carrier. No hazardous materials labeling is required. ADEQ has received verbal approval from DOT and UPS to ship decanted macroinvertebrate samples without having to meet hazardous materials shipping requirements because of the very limited quantities of isopropanol present in the decanted samples. Normal address labels are used for shipping. Enclose Chain of custody form with the samples.

All macroinvertebrate samples from a spring sample event are shipped to the laboratory in July of each year or as soon as practical after sampling.

The taxonomy laboratory verifies receipt of samples listed on the chain of custody form. The laboratory also refreshes the alcohol preservative.

3.15.1.8 Literature Cited

ADEQ, 2005. Biocriteria Program Quality Assurance Program Plan, revision D. ADEQ, Phoenix, AZ.

- **3.15.X.X Macroinvertebrate Taxonomy Laboratory Procedures**

The procedures followed at the taxonomic laboratory are not part of the field sampling procedures, however they are presented here for reference purposes. These procedures include sample processing, sorting, taxonomic identification levels, voucher specimens, and general quality control procedures.

1. Sample processing -Upon receipt of the samples, the laboratory will check and adjust the preservation in each sample, catalog the samples, check the attached inventory for accuracy, and sign the chain of custody papers. The consultant will then notify ADEQ of the receipt of samples, any damaged samples, or discrepancies between the inventory and actual sample labels.

2. Sample sorting - Samples must be sorted to separate the invertebrates from the sample matrix. The entire sample should be floated in water in a white plastic tray. Large debris is rinsed and removed from the sample until all organic matter and invertebrates are floated off the mineral residue. The mineral residue is then searched for stone-cased caddisflies and molluscs.

3. Sub-sampling and sorting - Arizona samples typically contain thousands of invertebrates and must be sub-sampled for results to meet a minimum count of 500-600 organisms. A Caton Tray is be used to randomly obtain fractions of the total sample from which all the invertebrates are removed and counted. Additional fractions are selected until the 500-600 target level is reached after which the number of squares subsampled are recorded. Terrestrial insects and non-benthic insects (e.g. corixidae, other swimmers, mosquitos, or surface tension dwellers) should not be included in the count. Additional fractions are examined if one fraction is dominated by a single species. After the target number of specimens has been achieved, the entire unsorted sample is scanned for large or rare taxa, which may aid in identification of smaller instars or may expand the taxa list for that sample. The remaining unsorted sample is re-preserved with 70% isopropanol in individual containers and archived at the laboratory for one year from the date of sample receipt, after which time the laboratory will contact ADEQ prior to disposal.

4. Sorting - The sorting of invertebrates from the sample matrix shall be performed by trained technicians, using dissecting scopes with a minimum magnification of 6X. After identifications have been made, the sorted specimens, including the separated Chironomidae, should be archived for one year or incorporated into the reference or voucher specimen set. The laboratory shall keep logs for each sample sorted, the fraction sorted, sample matrix problems, etc. in addition to bench sheets of the taxa identified in each sample.

5. Sorting efficacy - The laboratory shall check the sample residues to check for a sorting efficacy of 95% or better. A statement of sorting efficacy for the ADEQ batch of samples should be presented in the laboratory report.

6. Taxonomic identification - Invertebrate identifications shall be performed by a trained

and experienced taxonomist. The taxonomy contractor is responsible for obtaining the most accurate, consistently achievable identifications for ADEQ samples, using specialists as needed to obtain identifications to the general taxonomic levels listed in Table 3.15.X.X.

7. Reference specimens - A set of reference or voucher specimens shall be prepared from the batch of samples each year for incorporation into the reference specimen collection. Several specimens shall be preserved for each new taxon and the best or largest larval instars of other taxa shall be preserved to represent the taxa found that year and to update the historic reference collection at ADEQ. The taxonomist shall make recommendations for archiving any important specimens, if verification of identification by national specialists is required.

Table 3.15.X.X. ADEQ Taxonomic levels of identification for macroinvertebrates.

Invertebrate Group	Level of taxonomy required
Aquatic insects (except the family Chironomidae)	Genus (or species where consistently identifiable)
Chironomidae	Family
Semi-aquatic insects	Family
Arachnida (Mites)	Class
Cladocera, Copepoda, Ostracoda	Class
Amphipoda, Decapoda, Isopoda	Class
Nematoda, Nematomorpha	Phylum
Turbellaria	Class
Annelida	Class
Mollusca	Family or Genus

8. Lab Data Reports - Laboratory reports containing taxonomic identifications and counts for all samples for that year shall be submitted to ADEQ in electronic format. The electronic data shall be submitted in ACCESS database format or Excel spreadsheets formatted for database uploading. The Taxonomy Contractor shall perform quality control checks on the electronic data prior to submittal to ADEQ. The data set should contain at a minimum the StationID, waterbody name and location, habitat, collection date, complete taxa ID from phylum to lowest level ID, raw number of individuals, the portion of sample analyzed including field splits where applicable, and adjusted final counts, which are corrected for sub-sample size and field splits. A copy of the bench sheets used by the taxonomist to develop the raw counts per sample should also be submitted.

3.15.2 Arizona Indexes of Biological Integrity

3.15.2.1 Calculating the Arizona Indexes of Biological Integrity

The Arizona Indexes of Biological Integrity can be applied to macroinvertebrate taxonomic data generated by the sample collection procedures provided in this document. All the appropriate sample collection conditions must be met in order to calculate the IBIs for bioassessment purposes (ie. application of the narrative biocriteria standard). There are currently two Indexes; a cold and a warm water IBI. The following narrative provides the steps needed to calculate these Indexes from taxonomic lists and abundance data generated by taxonomy laboratories from the field collected macroinvertebrate samples.

1. Identify the appropriate reference community using the site elevation.

   - The warm water community is defined as being located below the 5000 foot elevation.
   - The cold water community is defined as being located above the 5000 foot elevation.

2. Calculate the macroinvertebrate metric values for the study sample following metric calculation procedures listed Figure 3.15.2.1a. Table 3.15.2.1a lists all the metrics used in both indexes and their definitions.

3. Calculate the metric percent of reference score using either the warm or cold water reference metric threshold values associated with that community type. Metrics required for each index and the associated threshold scoring values are listed in Tables 3.15.2.1b and 3.15.2.1c.

4. Calculate an average of the percent of reference scores for all metrics to produce the IBI score. An example of the scoring system is provided in Table 3.15.2.1d.

5. Determine assessment category for the IBI score from Table 3.15.2.1e.

3.15.2.2 Literature Cited

ADEQ, 2005. Biocriteria Program Quality Assurance Program Plan, revision D. ADEQ, Phoenix, AZ.

Figure 3.15.2.1a. Formulas for calculating macroinvertebrate metrics used in the cold water and warm water Indexes of Biological Integrity.

Use the following formula to calculate the metric score (percentage of reference) for sensitive metrics whose values decrease with disturbance. Apply this formula to the following metrics.

Metric Score = (Sample value / metric threshold value) * 100

1. Total taxa richness
2. Number of Ephemeroptera taxa
3. Number of Tricoptera taxa
4. Number of Diptera taxa
5. Number of intolerant taxa
6. Percent Ephemeroptera
7. Percent Plecoptera
8. Percent scrapers
9. Number of scraper taxa

Apply the following formulas to calculate the metric score (percentage of reference) for tolerant metrics whose values increase with disturbance.

1. Hilsenhoff Biotic Index

   Metric score = (10 – Sample value) / (10 – Metric threshold value) * 100

2. Percent dominant taxon

   Metric score = (100 – Sample value) / (100 – Metric threshold value) * 100

Table 3.15.2.1a Descriptions for the warm water and cold water metrics used in Arizona's IBIs.

Category	Metric	Definition	Expected Response to increasing disturbance
Richness measures	Total number of taxa	Number of different macroinvertebrate taxa	Decrease
	# Ephemeroptera taxa	Number of mayfly taxa	Decrease
	# Trichoptera taxa	Number of caddisfly taxa	Decrease
	# Diptera taxa	Number of true fly larvae.	Decrease
	# Intolerant taxa	Number of taxa having a tolerance value #3	Decrease
Composition measures	% Dominant taxon	Percent abundance of the single most abundant taxon.	Increase
	% Ephemeroptera	Percent abundance of mayflies, compared to total abundance of the sample	Decrease
	% Plecoptera	Percent abundance of stoneflies, compared to total abundance of the sample	Decrease
Tolerance measure	Hilsenhoff Biotic Index	Abundance-weighted average tolerance of assemblage	Increase
Trophic measures	% Scrapers	Percent abundance of the scraper functional feeding group, compared to total abundance of the sample	Decrease
	# Scraper taxa	Number of taxa in the scraper functional feeding group	Decrease

Table 3.15.2.1b. Reference scoring thresholds for Warm Water metrics, used in the Arizona warm water Index of Biological Integrity.

Metric	Metric threshold value
Total taxa	37
Trichoptera taxa	9.0
Ephemeroptera taxa	9.0
Diptera taxa*	10.0
Scraper taxa	7.0
Percent scraper	23.7
Percent Ephemeroptera	70.0
Percent Dominant Taxon	19.1
Hilsenhoff Biotic Index	4.89

* Appropriate taxonomic effort is to genus for insects and to family for midges.

Table 3.15.2.1c. Reference scoring thresholds for Cold Water metrics, used in the Arizona cold water Index of Biological Integrity.

Metric	Scoring threshold
Total taxa	38
Diptera taxa*	11
Intolerant taxa	6
Scraper taxa	11
Percent scraper	45.1
Percent Plecoptera	19.1
Hilsenhoff Biotic Index	4.23

* Appropriate taxonomic effort is to genus for insects and to family for midges.

Table 3.15.2.1d. Example of the ADEQ Warm Water Index of Biological Integrity scoring system; Sycamore Creek near Round Valley bridge (Hwy 87) collected during spring 1995.

Metric	Metric Value	Metric Score (compared to warm water reference scoring threshold)
Total taxa	24	65
Trichoptera taxa	6	67
Ephemeroptera taxa	5	56
Diptera taxa	7	70
Scraper taxa	3	43
Percent scraper	20.3	86
Percent Ephemeroptera	26	37
Percent Dominant Taxon	41	73
Hilsenhoff Biotic Index	5.73	84
Index Score (average of all Metric Scores)		65 Attaining

Table 3.15.2.1e. Assessments based on ADEQ macroinvertebrate IBI scores.

Macroinvertebrate bioassessment result	**Index of Biological Integrity Score**		**Assessment**
	Cold water	**Warm water**	
Greater than the 25th percentile of reference condition	≥ 52	≥ 50	Attaining
Between the 10th and 25th percentile of reference condition	46 – 51	40 – 49	Inconclusive
Less than the 10th percentile of reference condition	≤ 45	≤ 39	Impaired